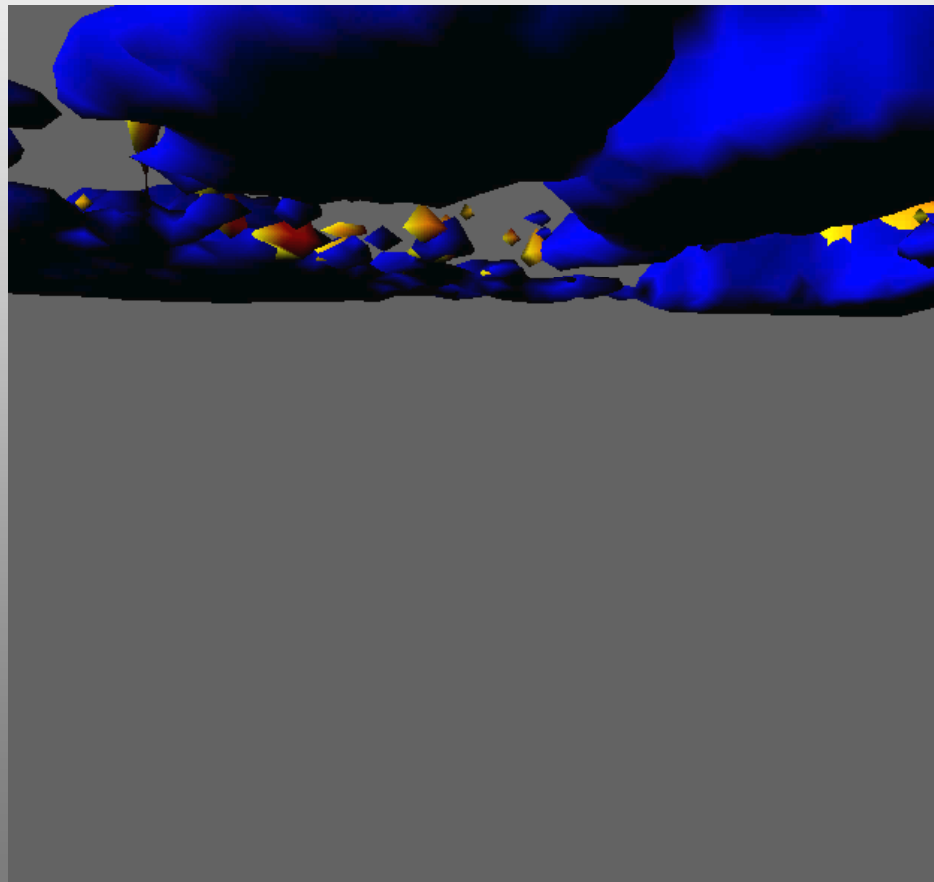


Biofilms

The Surface Associated Microbial World



Bacterial biofilms are everywhere

Biofilm: Bacteria attached to a surface encased in a matrix of extra-cellular polymeric substances (EPS)

99% of all bacteria in nature are enclosed in biofilms

From single cells to organized multi-cellular mature biofilms

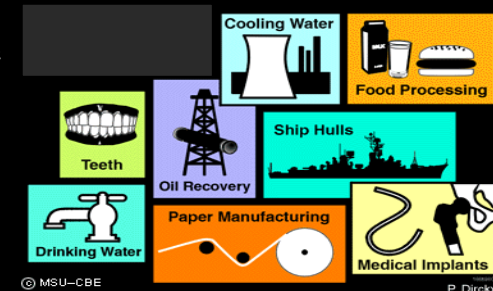
Where do we find biofilms?

- ♦ Fresh-water reservoirs
- ♦ The sea
- ♦ Soil particles
- ♦ Teeth
- ♦ Water pipes, water tanks and fermentores
- ♦ On internal bio-surfaces of humans and animals
- ♦ In cystic fibrosis, on prosthetic devices, etc.
- ♦ On food
- ♦ Basically any place that supports life

....AND IN THE LAB UNDER CONTROLLED CONDITIONS



Jurassic oolitic microbial mat community with fungal networks (200 million years old)



© MSU-CBE

P. Dirckx

In addition to the studies of bacteria in test tubes, it is important also to investigate the bacteria when growing as biofilms.

Why, because they here in many cases perform in a very different way

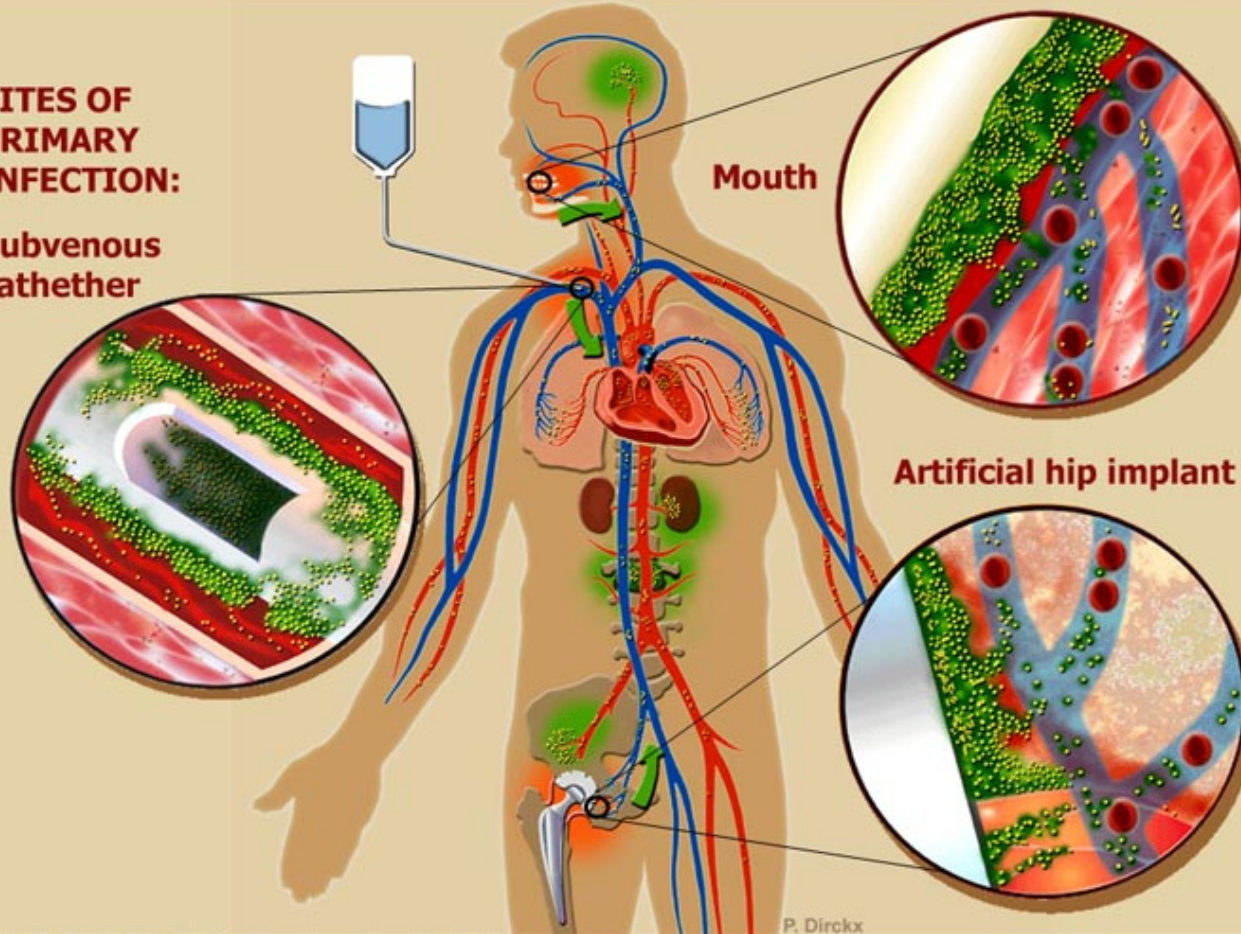
Sites of **Primary** and **Secondary** Biofilm Infection

**SITES OF
PRIMARY
INFECTION:**

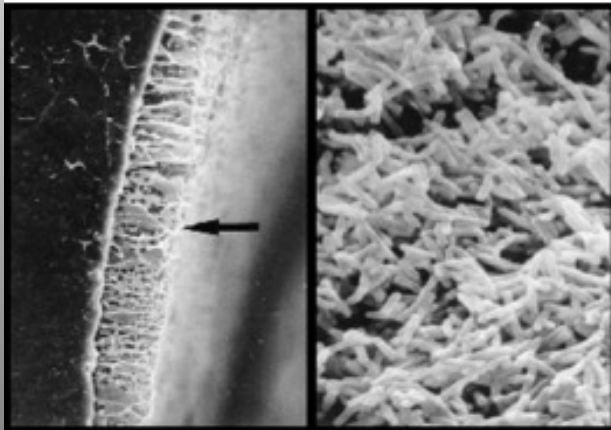
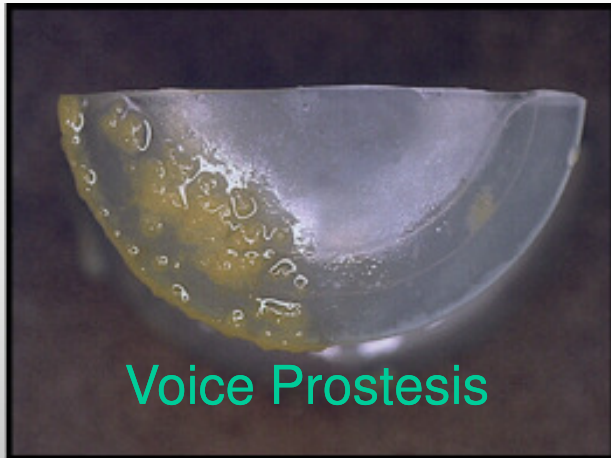
**Subvenous
cathether**

Mouth

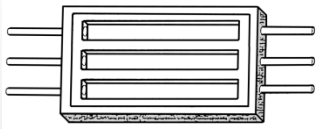
Artificial hip implant



Medical relevant biofilms



The laboratory biofilm tool-box



- Flow chamber system for biofilm cultivation

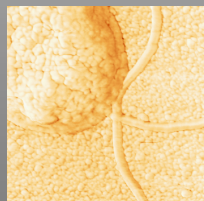
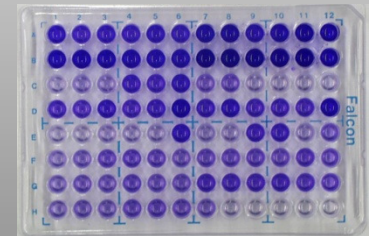
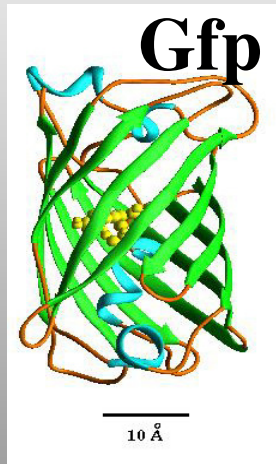
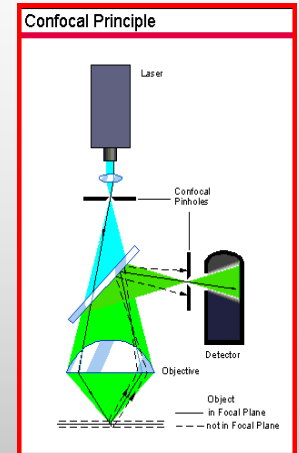
- GFP – Green Fluorescent Protein

- SCLM – Scanning Confocal Laser Microscope
 - Atomic force microscopy and SEM

- Microtiter assay for biofilm formation

 - The robot and colony picker

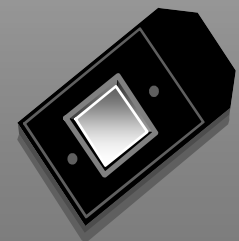
- The Cell sorter (FACS)



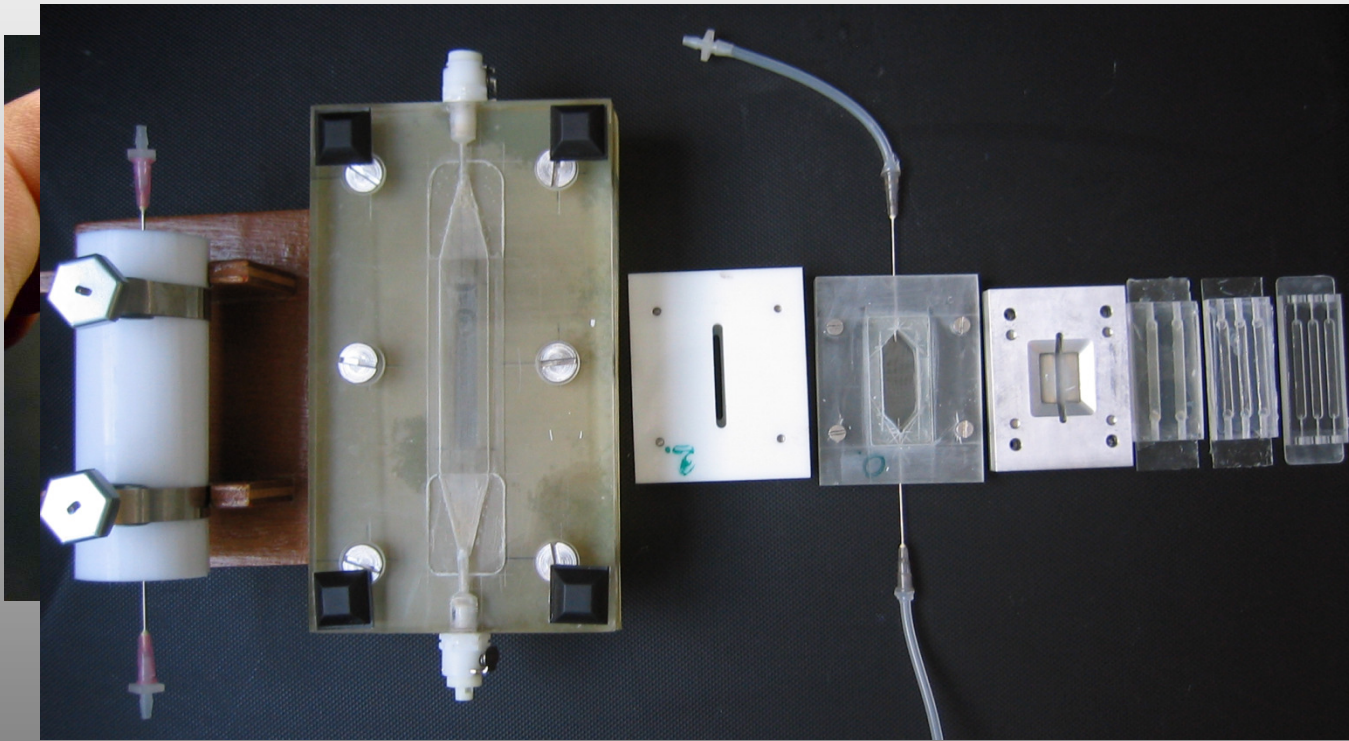
- Micro array analyses

- FISH – Fluorescent *In Situ* Hybridisation

- COMSTAT



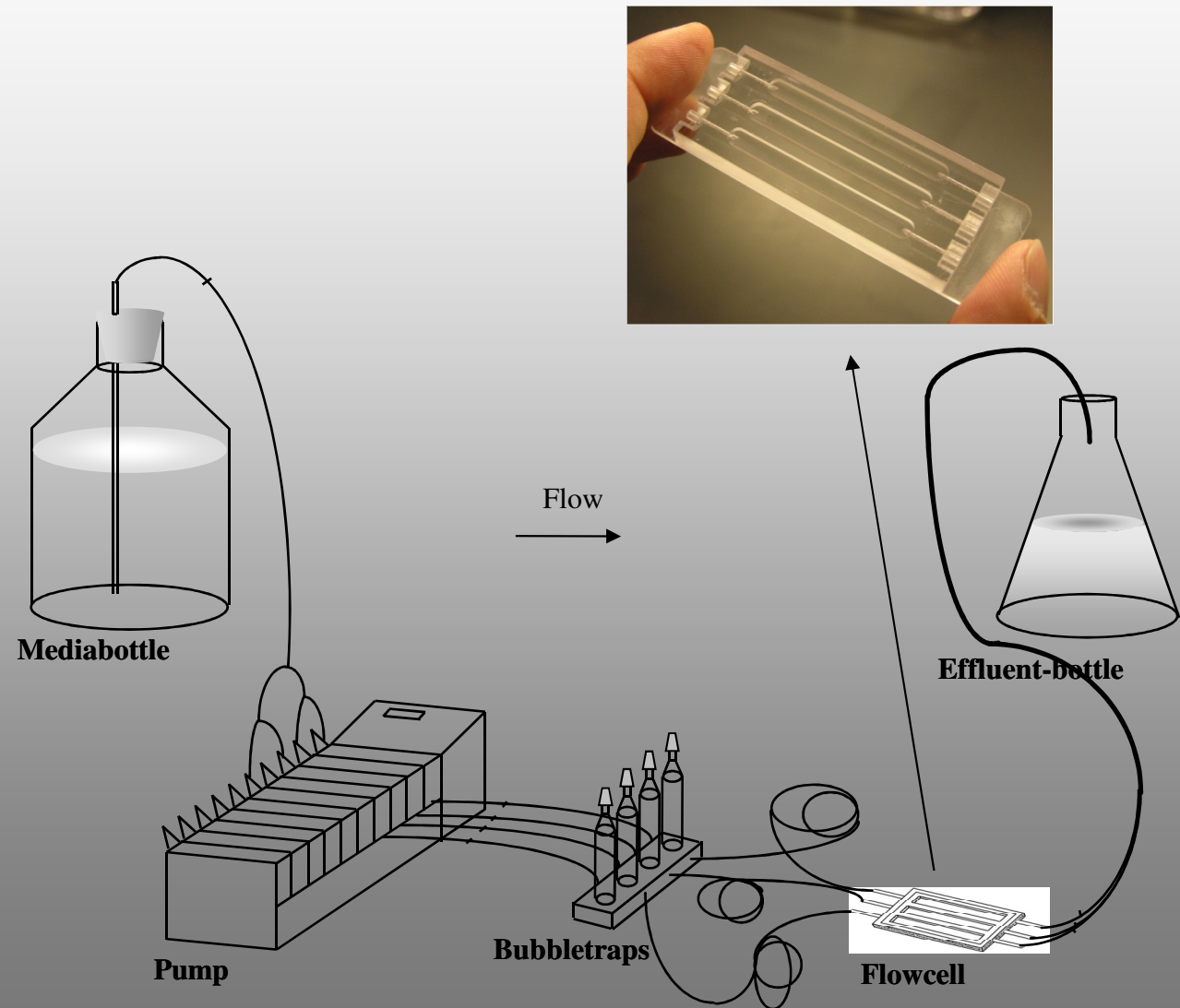
The DTU flow cell system



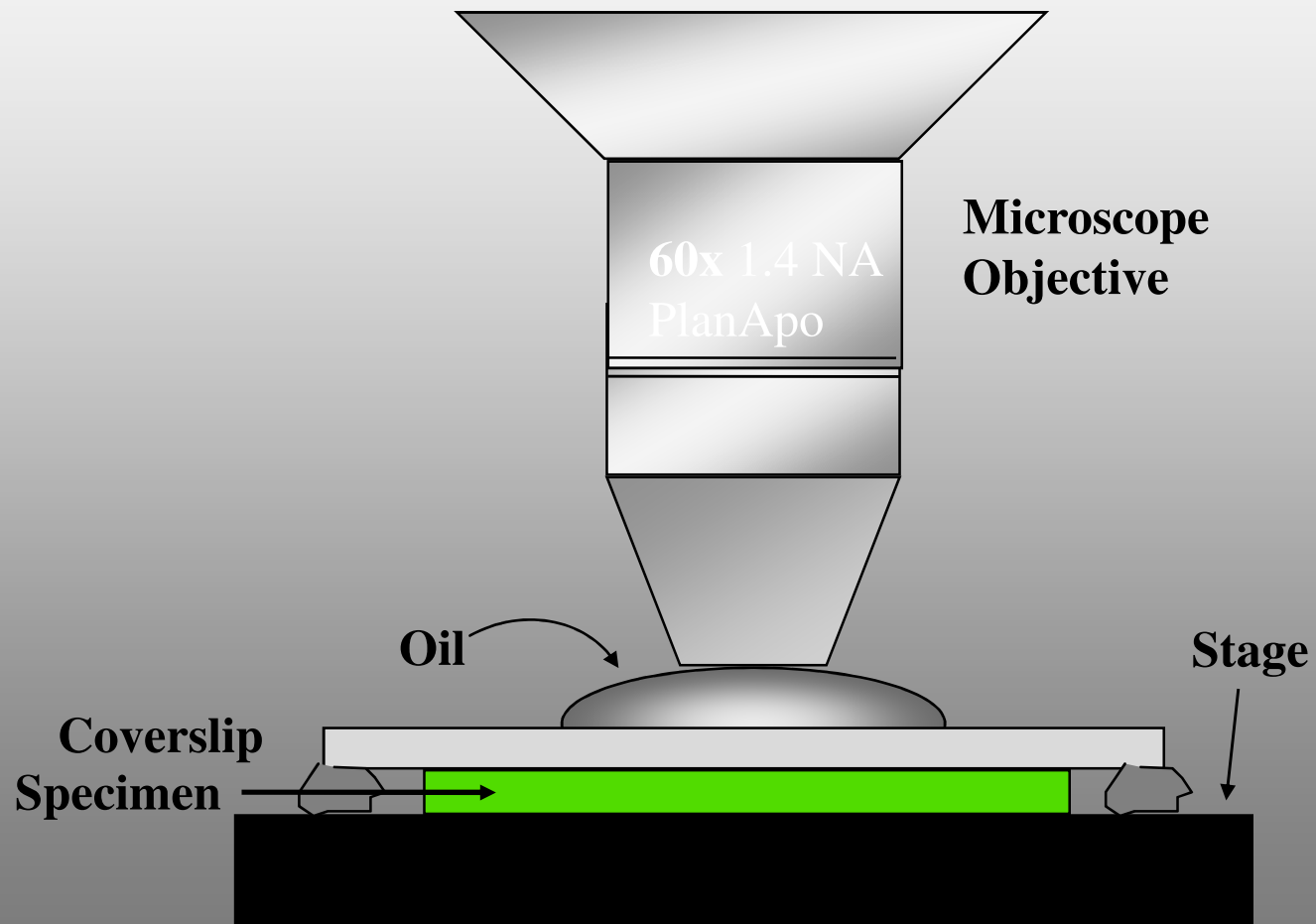
The Flow Chamber System

**The standard set-up
at DTU for hydro-
dynamic biofilm
development.**

**The flow-cells are
mountable directly
on the Confocal
Microscopes for in
situ investigations
(Zeiss LSM510
or Leica DMRXA)**



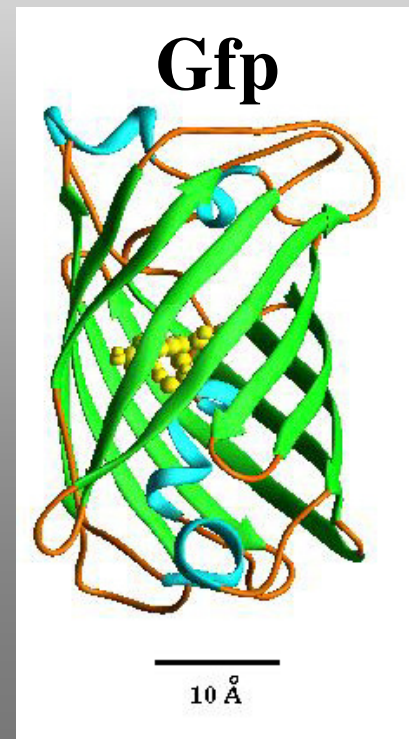
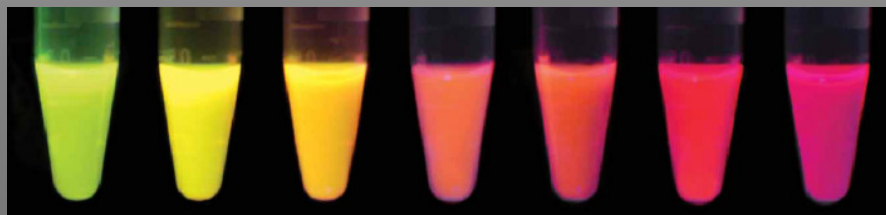
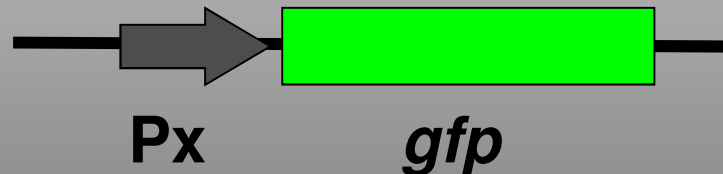
Microscope Objectives



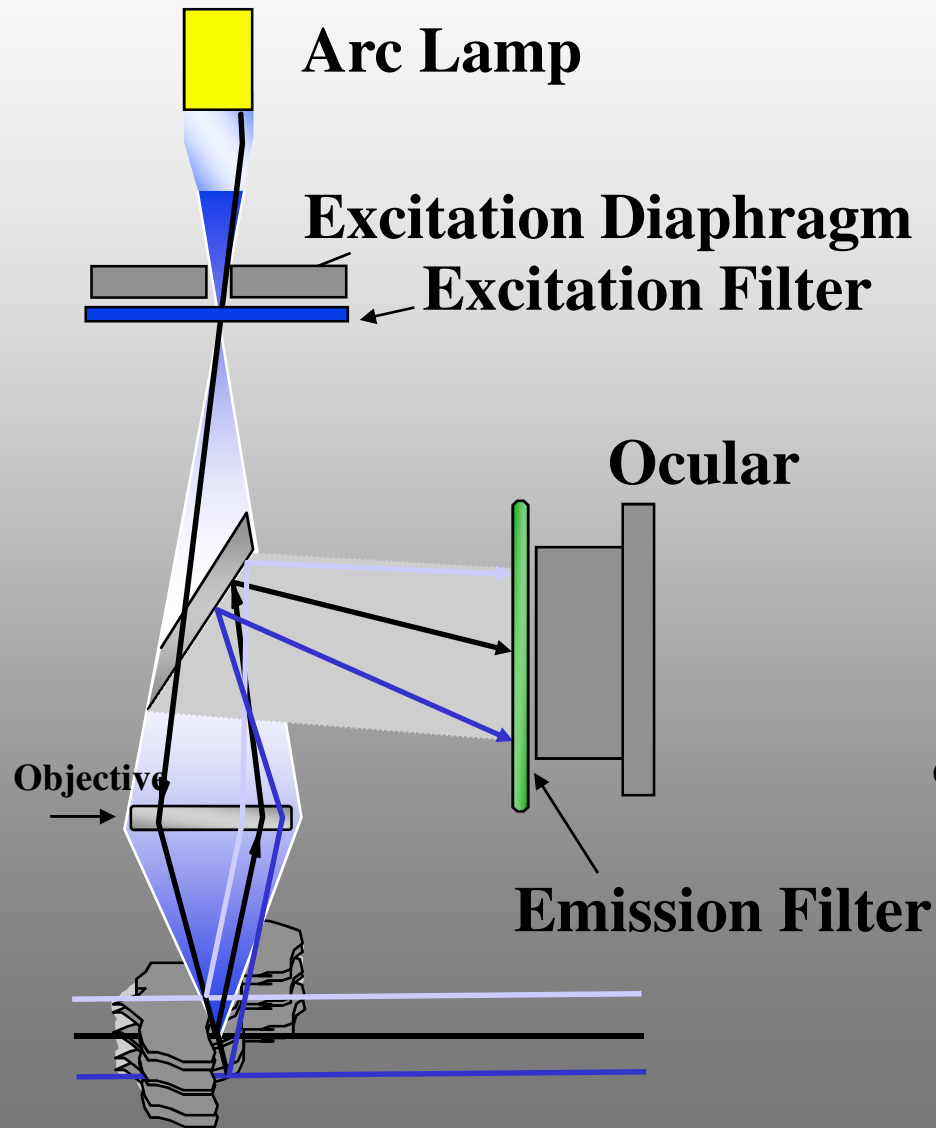
Green fluorescent protein (Gfp)

Tagging and reporter cassettes with Gfp, Yfp, Cfp, and Rfp genes are available

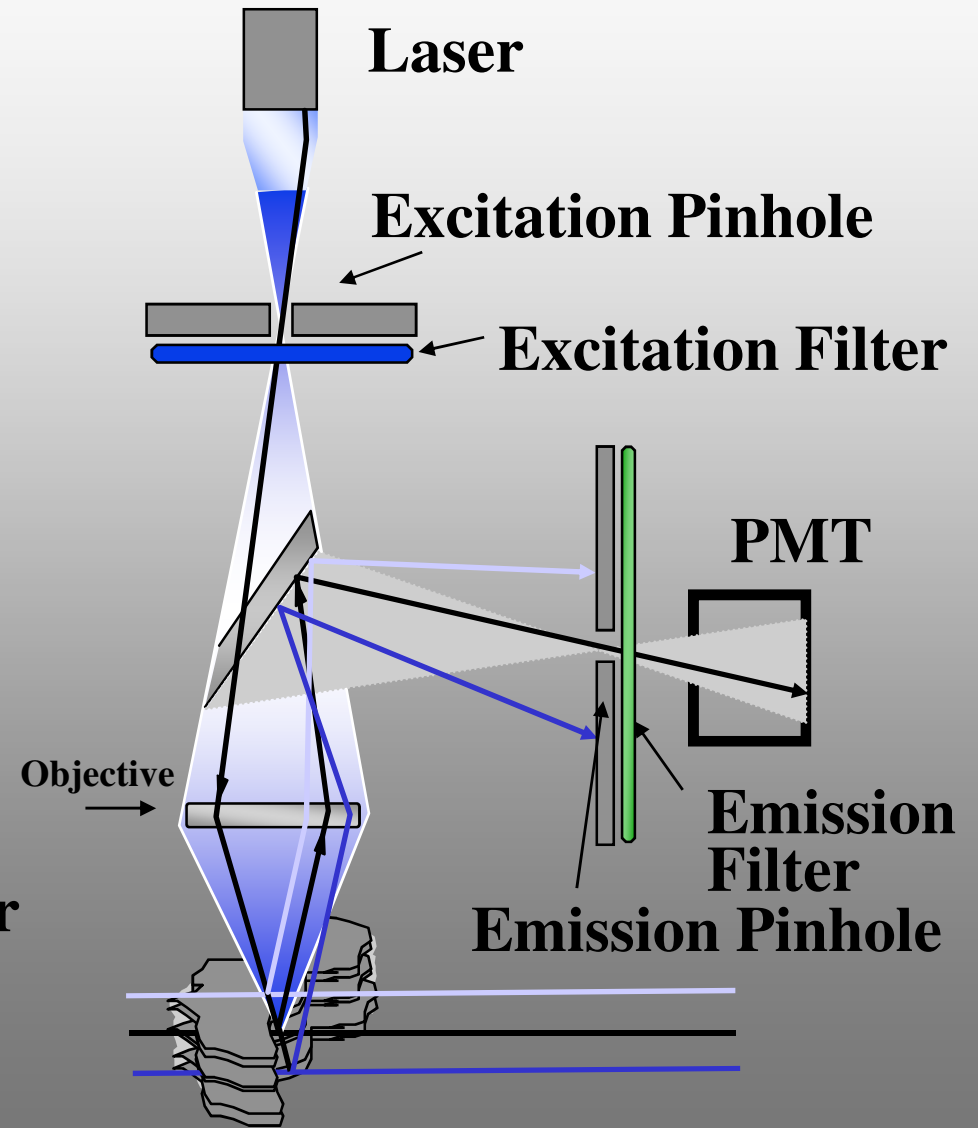
- ♦ Isolated from the jelly fish *Aequorea victoria*
- ♦ Excellent for studying gene expression in vivo
- ♦ Molecular reporter cassettes for visualization of e.g. substrates and growth activity
- ♦ Requires only oxygen to mature
- ♦ Works in bacteria, yeast, plants and mammals



Fluorescent Microscope



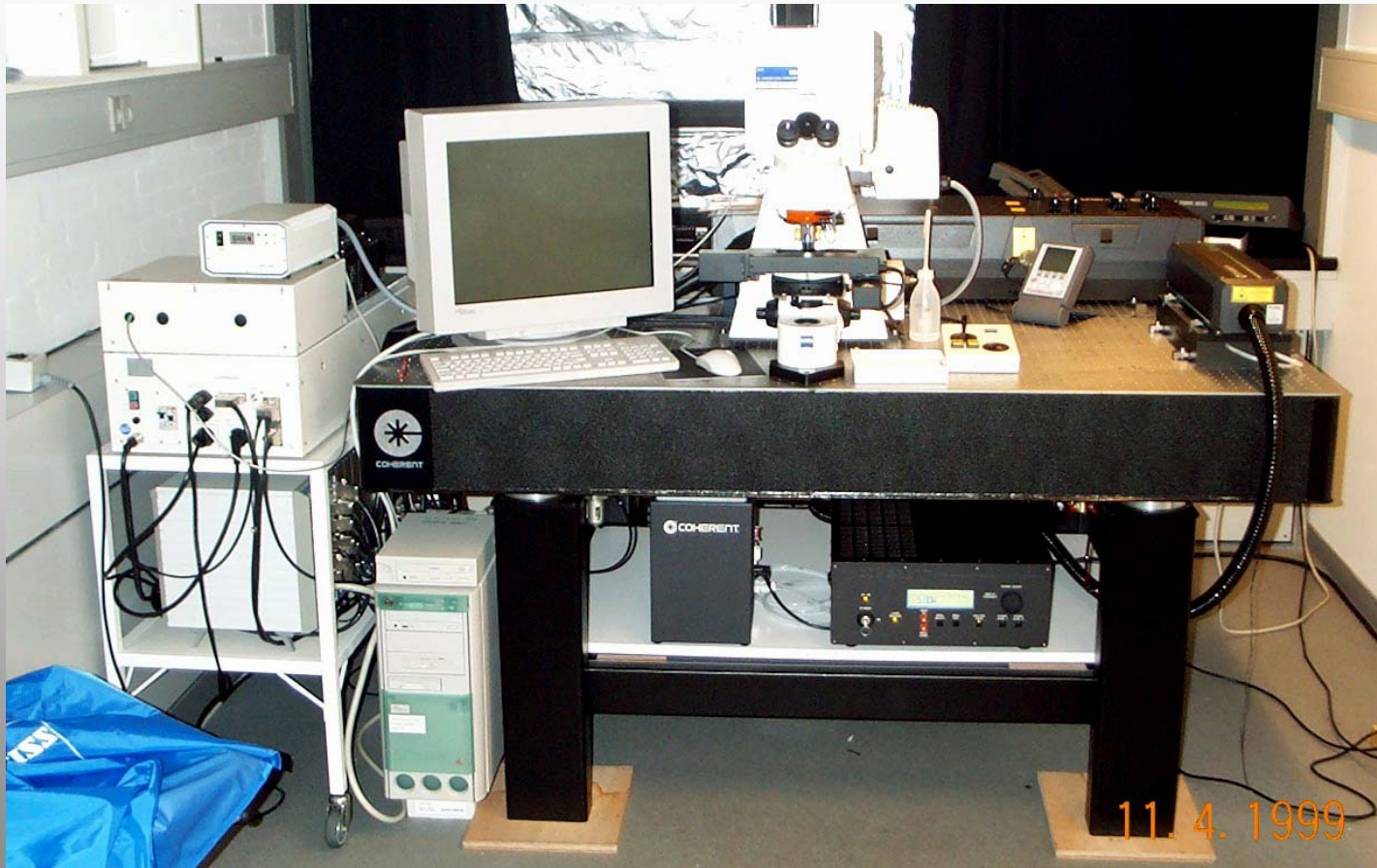
Confocal Microscope

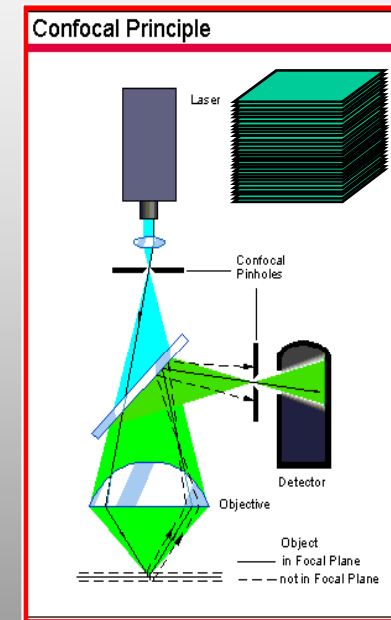


Benefits of Confocal Microscopy

- Reduced blurring of the image from light scattering
 - Increased effective resolution
 - Improved signal to noise ratio
- Clear examination of thick specimens
 - Z-axis scanning
- Magnification can be adjusted electronically

Multiphoton facility on the Zeiss confocal





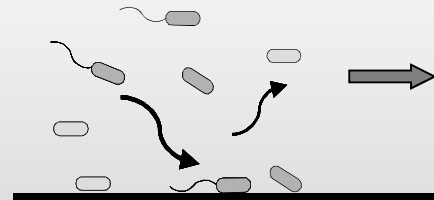
μm scale

The Biofilm Development Cycle

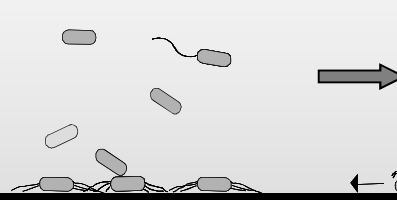
In hydrodynamic conditions biofilm development depends on *adhesive forces*.

At the substratum bacterial motility may have significant impacts on structure development

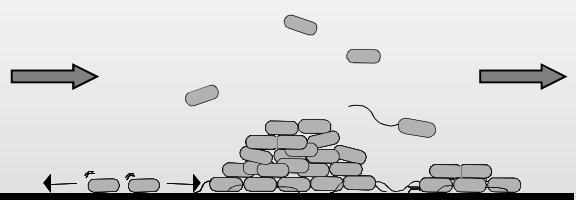
1) Reversible attachment



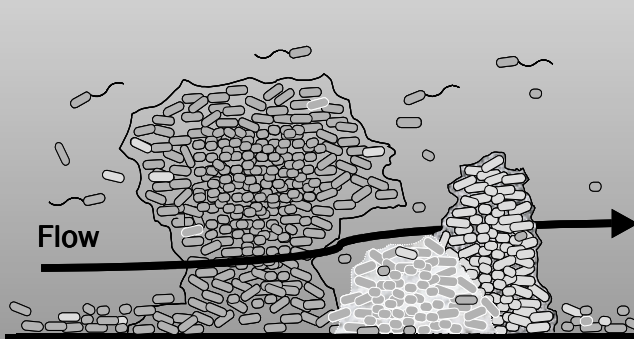
2) Irreversible attachment



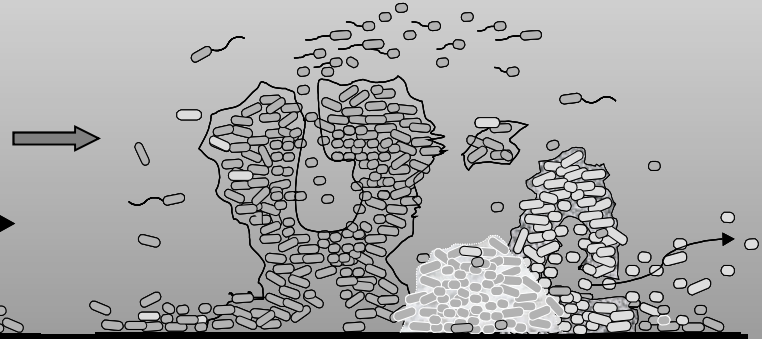
3) Cell proliferation



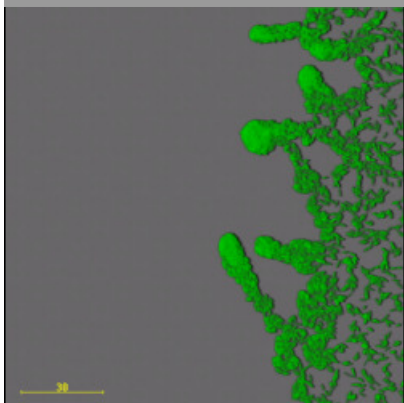
4) Biofilm maturation



5) Disintegration



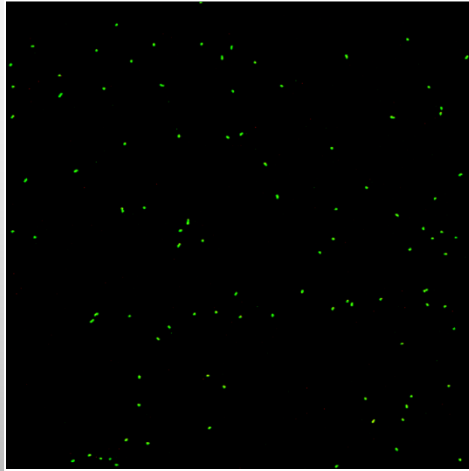
- **Colonizers** – able to attach to surface
- **Stickers** – able to form micro-colonies
- **Twitchers** – able to migrate on surface
- **Swimmers** – able to move in water column
- **Swarmers** – able to spread on conditioned surface



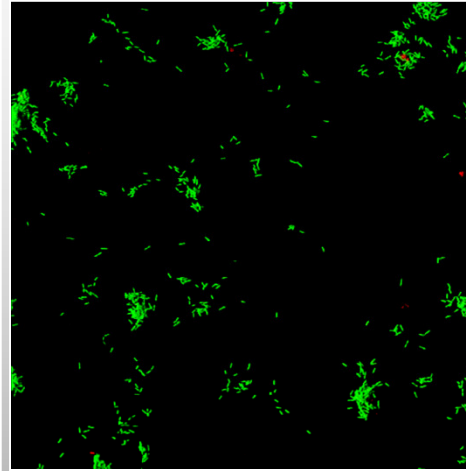
Biofilm Development

Biofilm development
as seen through the
scanning confocal
laser microscope

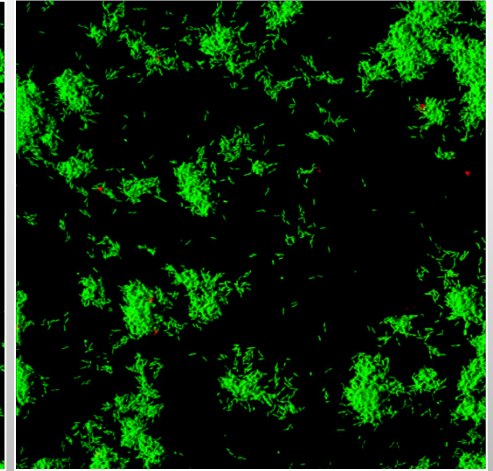
1) Reversible attachment



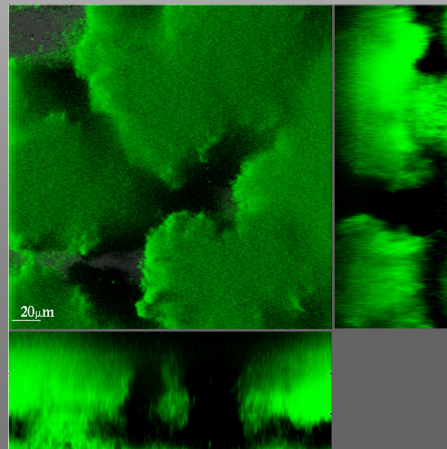
2) Irreversible attachment



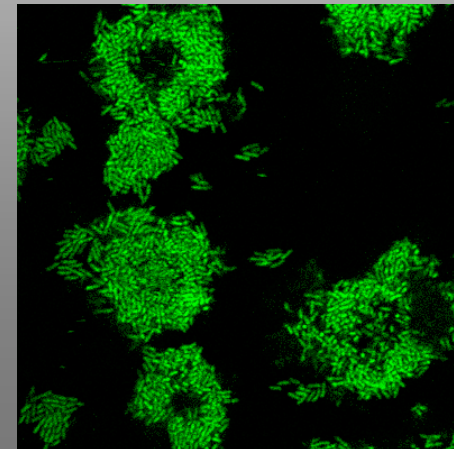
3) Cell proliferation



4) Biofilm maturation

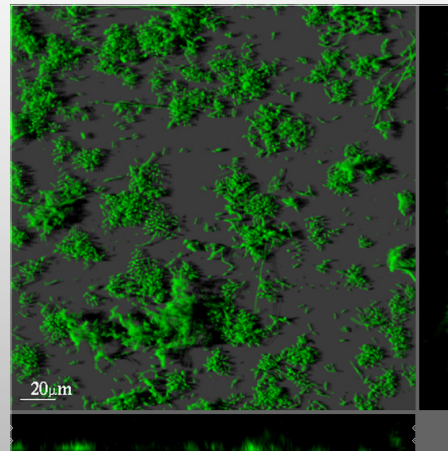


5) Dissolution

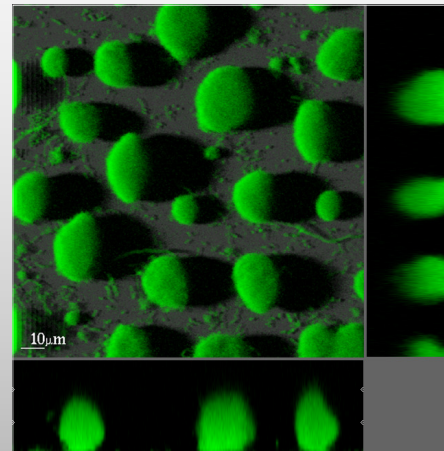


The Zoo: Mature Flow-Chamber Biofilms

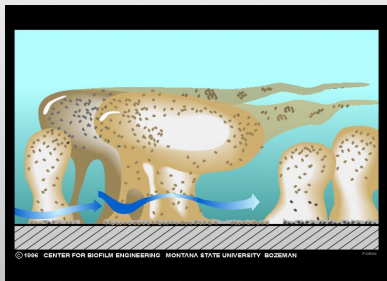
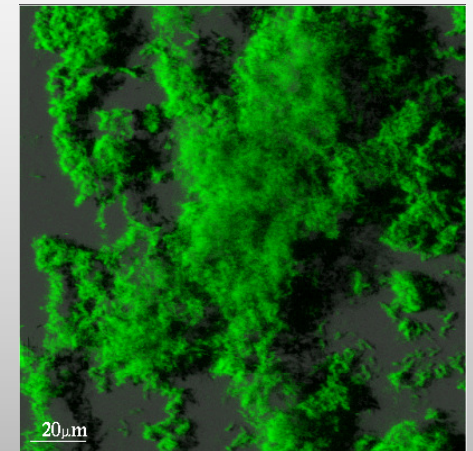
E. coli F-



Acinetobacter spp.

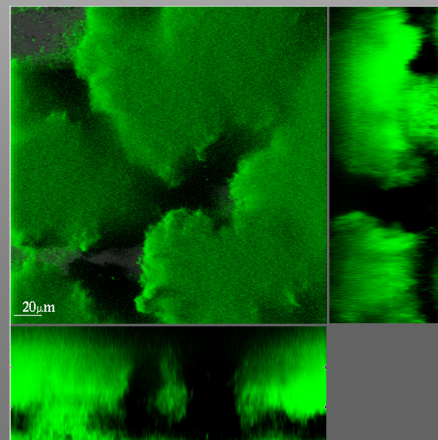


P. putida

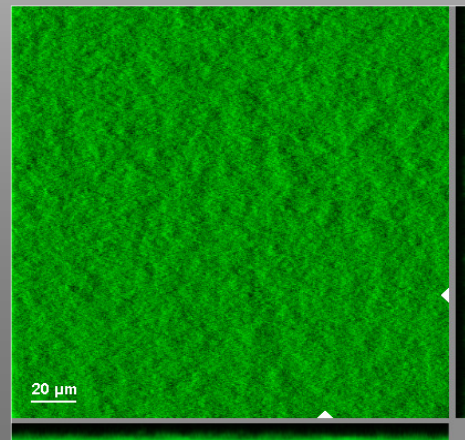


The *P. aeruginosa* model

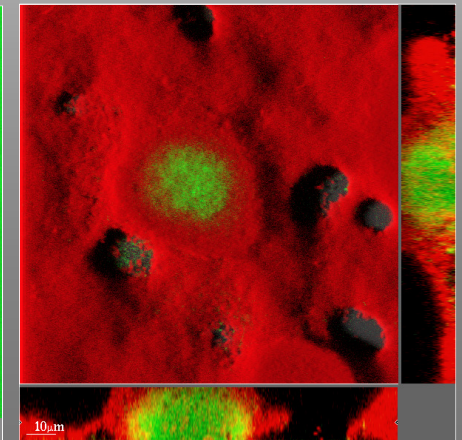
E. coli F+/
P. aeruginosa (1)



P. aeruginosa (2)



P. syringae



No indication of
consensus structure
of mature biofilms

Biofilm Development

Biofilm structure/function development is influenced/determined by:

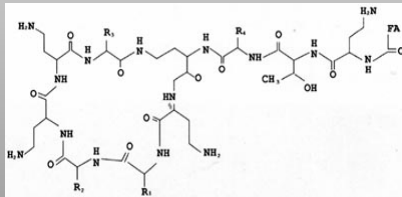
- **The nutritional conditions (cf. Cristian Piciooreanu)**
- **The characteristics of cell surface adhesives**
- **The motility properties (swimming, twitching...)**
- **Development of distinct sub-populations**
- **Matrex production (Polysaccharides, proteins, ekstracellular DNA...)**
- **Cell to cell communication (quorom sensing...)**
- **Exchange of genetic information (conjugation, transduction)**

In addition, specific dissolution features may impact the structural properties of biofilms

And the major factor driving biofilm development:

Food acquisition

Subpopulation development and differentiation in *E. coli* biofilms

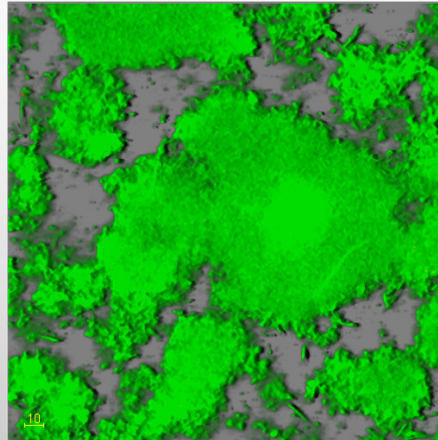


Colistin

- *E. coli* as the model organism
 - identification of specific subpopulations by antibiotic treatment of biofilms
- Colistin (a polymyxin) as the model antibiotic
 - Mode of action: Bactericidal, binds to and interferes with the cytoplasmic membrane
- In situ monitoring of live and dead cells in biofilms by use of the BacLight Live/dead kit
- Colistin acts directly on the membrane, the target for the BacLight indicator

Antibiotic activity in *E. coli* Biofilm

Gfp tagged micro-colony.
Before treatment with colistin.

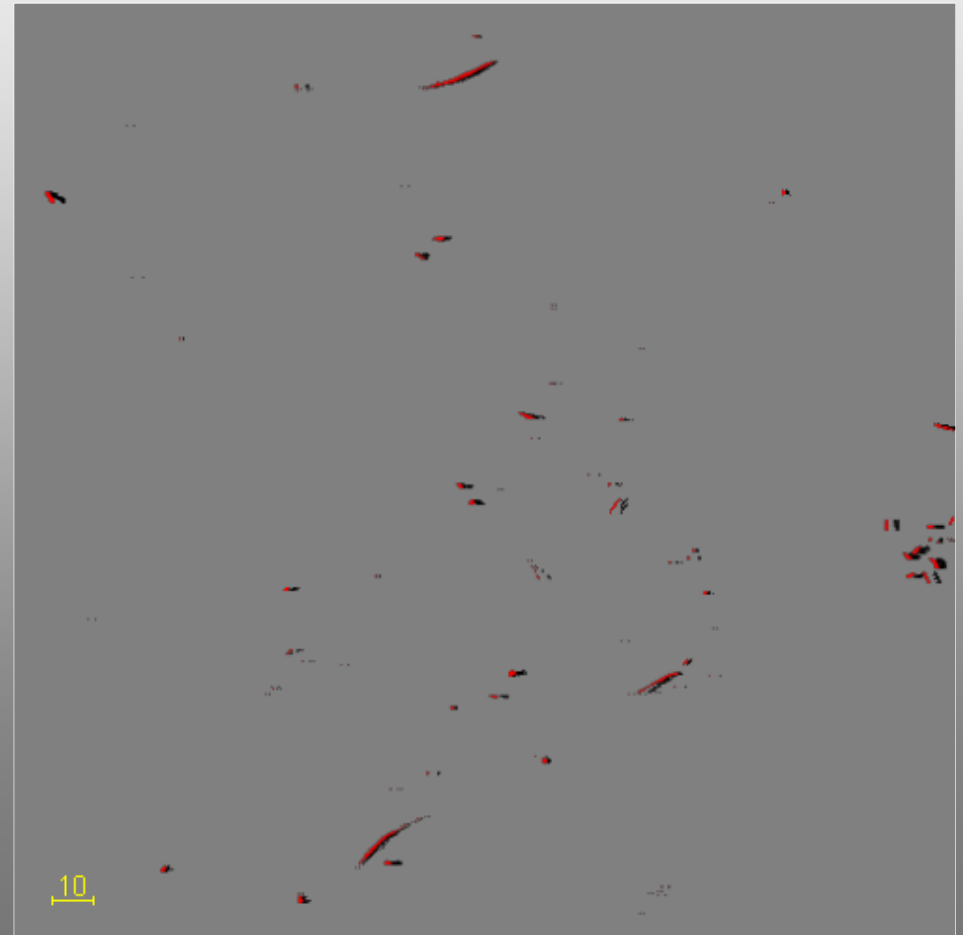


Dynamics of killing (20hrs)

Treatment with colistin simultaneous staining of dead cells with Propidium Iodide

Green cells are alive

Red cells are dead



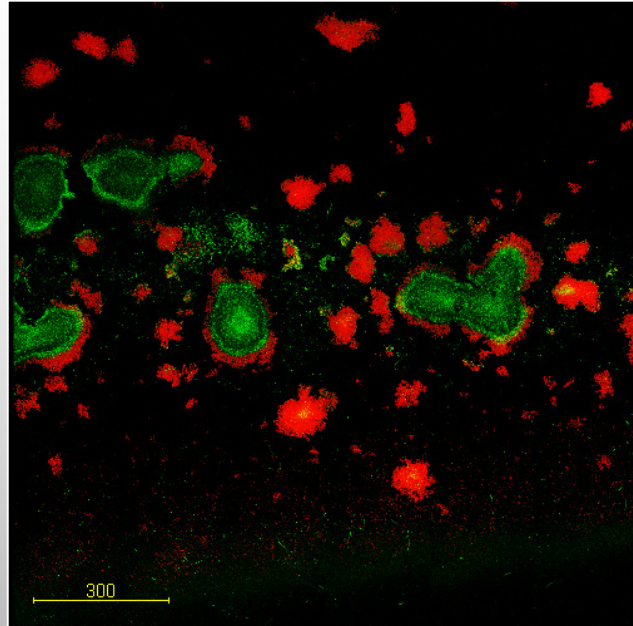
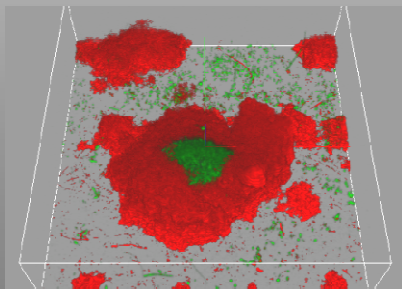
The killing activity of colistin moves from the edge of the micro-colonies to the inner parts

No detachment of dead cells

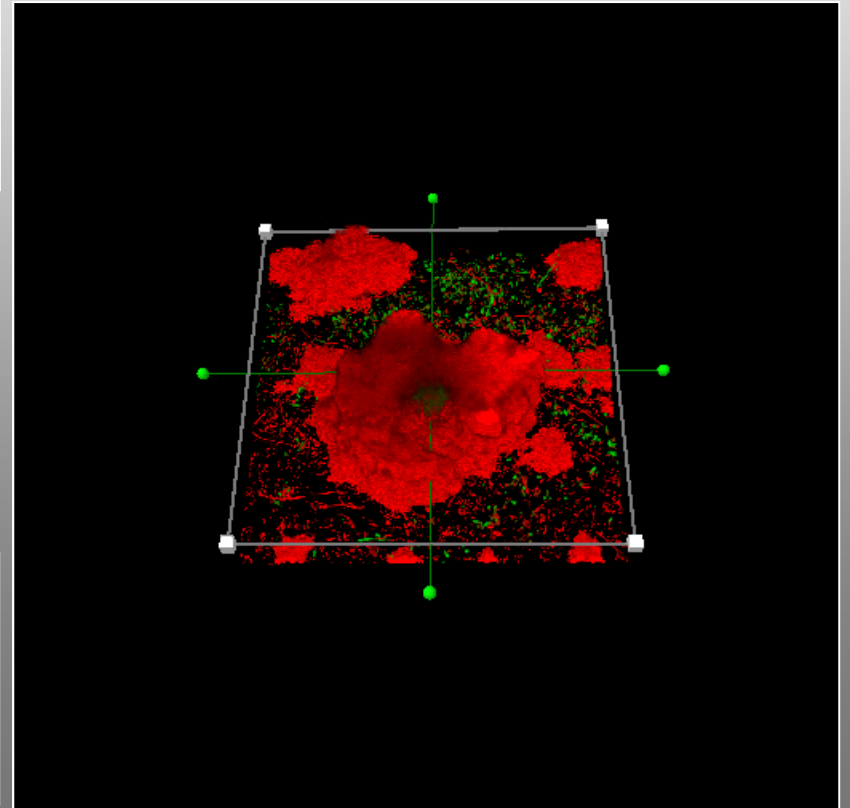
Antibiotic tolerance in *E. coli* biofilm

Gfp labeled cells

Colistin treatment for 24 hrs followed by staining with propidium iodide



Overview of biofilm structure (10x)



Cell differentiation result in subpopulations with increased tolerance to antibiotic

- Imaris, an image treatment and projection software
- Demonstration